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Applicant:	KONO, Rikako et al.	Conf.:	5915
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For:	LARGE CONDUCTANCE CALCIUM-ACTIVATED K CHANNEL OPENER		

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hajime Aihara, declare the following.

I am fully knowledgeable of the disclosure of the above-identified application and the field of art of the present invention. I have read and understand the Office Action dated June 1, 2004 and the references cited therein, Leonardi et al. (U.S. Patent 6,440,963) and Hongu (WO 02/083111).

Attached is a paper titled "Investigation on contribution of COX inhibiting action and BK channel opening action in the acid-induce pollakiuria model rats" which was written by me. The experiments described in this paper were either performed by me

or were performed under my direct supervision. Furthermore, all statements made in the paper I adopt as my own.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Hajime Aihara
Signature

Oct. 7, 2004
Date

Hajime Aihara
Name in print

Investigation on contribution of COX inhibiting action and BK channel opening action in acetic acid-induced pollakiuria model rats

[Abstract]
Using acetic acid-induced pollakiuria model rats, actions of Valdecoxib (selective COX2 inhibitor, also having BK opening action), NS-398 (selective COX2 inhibitor having no BK opening action) and Indomethacin (non-selective COX inhibitor having no BK opening action) were investigated. In the acetic acid-induced pollakiuria model, Valdecoxib and Indomethacin clearly showed a bladder capacity-increasing action, but NS-398 did not show such an action (administration doses are all 3 mg/kg i.d.). An action of Indomethacin was not affected by pretreatment of iberiotoxin (1 ng/kg/min i.v. infusion) which is a BK channel inhibitor, but the bladder capacity-increasing action of Valdecoxib was diminished. These results suggest that the COX1 inhibiting action and BK channel opening action are mainly participated in bladder capacity-increasing effects of these compounds in the present model.

[Abbreviation]

COX :Cyclooxygenase
BK channel :Large conductance calcium-activated potassium channel

[Method]
Female SD rats (9 weeks old, 200 to 280 g) were used for the experiments. 1.2 g/kg of urethane was subcutaneously administered to rats to induce anesthesia. After fixing in supine position, the bladder was exposed by an incision in the midline. The catheter was inserted into the bladder and connected via T-tube to a pressure transducer (TP-400T, Nihon Kohden, Japan) for the measurement of intravesical pressure and microinjection pump. Ureters were cannulated for excretion of urine. The tube was installed in the external urethral orifice and used for the measurement of micturition volume. The excretion fluids from the external urethral orifice were collected by small cup, which connected force-displacement transducer. The catheter for the administration of a drug was inserted into the duodenum and fixed. The cannulae were placed in femoral veins for the infusion of saline or iberiotoxin.

The right carotid artery was cannulated and the one end of the catheter was connected to a pressure transducer for monitoring systemic arterial pressure. Heart rate was obtained from systemic arterial pressure waves using heart rate counter

After continuous infusion of the physiological saline into the bladder for one hour, the physiological saline was changed to a physiological saline containing 0.2% of acetic acid (pH about 3), and after the blood pressure, heart rate and urination kinetics were stabilized, continuous infusion of a vehicle (physiological saline solution) or iberiotoxin was started. After urination kinetics were stabilized, the drug was administered intraduodenally and a bladder capacity (BC) was monitored as a measurement parameter. The results of BC were shown as an average value for 20 minutes, and expressed as a percentage of pre-drug value.

[Results and consideration]

It has been reported that a COX inhibitor and a BK channel opener act on overactive bladder. On the other hand, we found out that the compounds such as celecoxib and valdecoxib among the COX inhibitors have a BK channel opening action but that indomethacin or NS-398 does not have a BK opening action. Thus, it has not yet been clarified at present, which the BK channel opening action and the COX inhibiting action contributes to the action on the bladder and how it acts. Therefore, actions of the COX inhibitors having various profiles were investigated by using an acetic acid-induced pollakiuria model and we tried to clarify the cause of the action on the bladder.

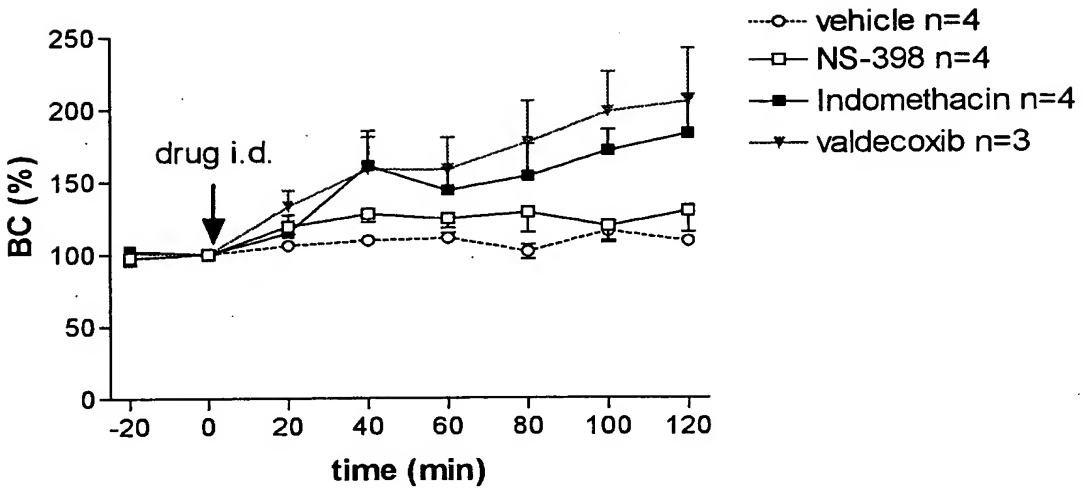

Figure 1 Effect of several COX inhibitors on bladder capacity (BC) in acetic acid-induced urinary frequency model.

Actions of the respective COX inhibitors on the bladder capacity in the rat acetic acid pollakiuria model are shown in Figure 1. The respective compounds were tested by administering them to the duodenum in an amount of 3 mg/kg which is considered to show sufficient plasma concentration as a COX inhibiting action. As a result, NS-398 which is a selective COX2 inhibitor did not show a bladder capacity-increasing action in this model. On the other hand, indomethacin which is a non-selective COX inhibitor showed clear bladder capacity-increasing action. Valdecoxib which is a selective COX2 inhibitor and has a BK channel opening action showed potent bladder capacity-increasing action similarly.

Whereas NS-398 and valdecoxib are the both selective COX2 inhibitors, valdecoxib has a BK channel opening action different from NS-398. Thus, in order to examine whether the action of valdecoxib is mainly based on the BK channel opening action or not, actions of indomethacin and valdecoxib were examined under intravenous continuous infusion of iberiotoxin which is a BK channel inhibitor.

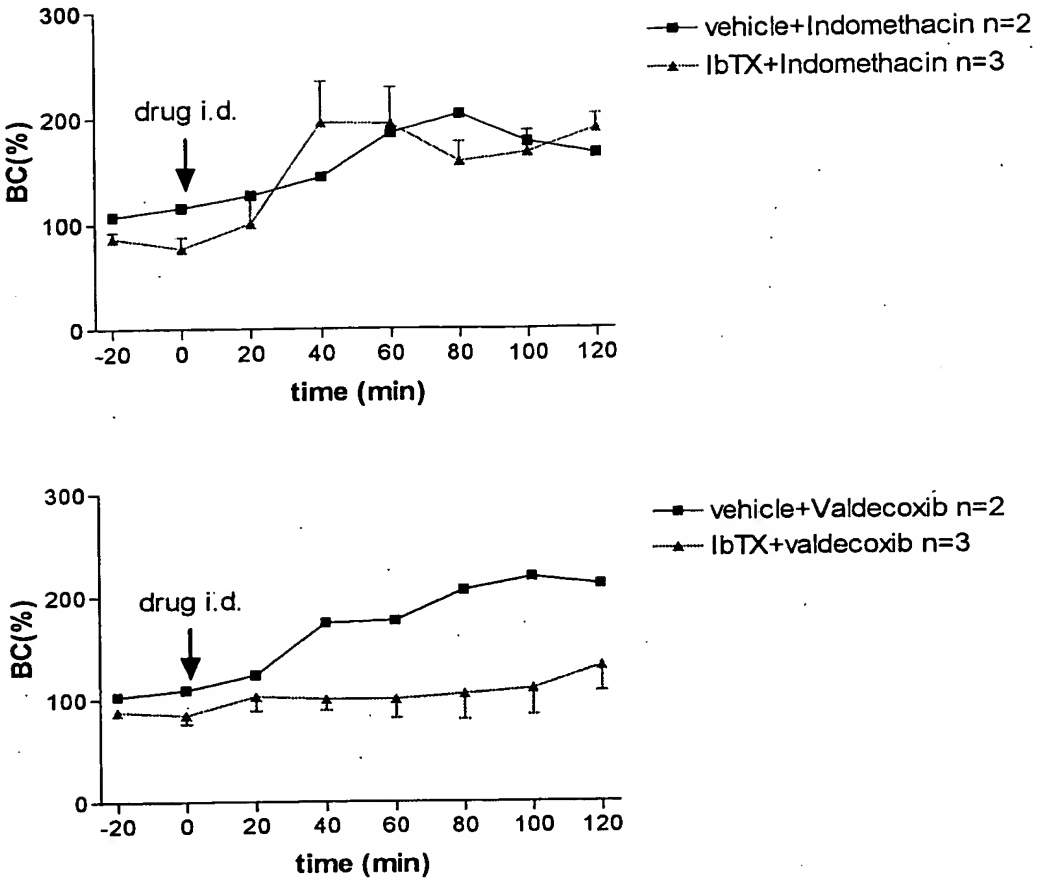

Figure 2 Effect of COX inhibitors with or without iberiotoxin on bladder capacity (BC) in acetic acid-induced urinary frequency model.

In Figure 2, actions of indomethacin and valdecoxib in the presence of iberiotoxin are shown. In the presence of iberiotoxin (1 ng/kg/min, i.v. infusion), the bladder capacity was lowered about 20% than the case of the acetic acid stimulation alone. The bladder capacity-increasing action of indomethacin was not affected by the presence of iberiotoxin, but the bladder capacity-increasing action of valdecoxib was diminished in the presence of iberiotoxin.

From the fact that indomethacin, which is a non-selective COX inhibitor having no BK channel opening action shows clear bladder capacity-increasing action, it is suggested that COX is participated in this model. On the other hand, according to the investigation of NS-398 and valdecoxib both having a COX2 selective-inhibiting action, NS-398 did not show any action, but valdecoxib showed a potent bladder capacity-increasing action. Since valdecoxib has a BK opening action, and the

bladder capacity-increasing action of valdecoxib was diminished in the presence of iberiotoxin, it can be considered that the action of valdecoxib is based on the BK channel opening action, and participation of COX2 in this model seems to be little. From the above, a possibility that COX1 and BK channel are participated in the bladder capacity-increasing action of various kinds of COX inhibitors in this model is suggested.